



User manual

NBB®-P (Product No. 2.04716.462)

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1. Information

Powder for production of NBB®-Broth and NBB-Agar® for the detection of Beer spoiling bacteria (esp. *Lactobacilli*, *Pediococci*, *Pectinatus* and *Megasphaera*) in filtered beer samples, water samples, yeast samples as well as for membrane and swab samples.

NBB®-Powder (NBB®-P) is a powder, which dissolved in your own beer, will adapt the selectivity of NBB®-Media onto the demand of your products.
To obtain NBB®-B dissolve the powder in beer and demineralised water. For NBB®-A add Agar-Agar additionally to the broth.

2. Handling

Required Material

Microbiological workbench
Magnetic stirrer with magnetic stir bar
Conical flask
Clean laboratory bottles with adequate screw tops qualified for temperatures of 120°C/248°F and higher
Distilled or demineralised water (see ISO11133)
Degased beer
pH-meter with temperature compensator
Lactic acid (90%) or NaOH for pH-adjustment
Autoclave

Additional for production of NBB®-B:

Sterile swing stopper sample bottle (50 mL) or other suitable flask
Sterile test tubes with screw caps or porous stoppers

Additional for production of NBB®-A:

Agar-Agar
Water bath (max. temp. 95°C/203°F)
Petri dishes
Sterile swabs or inoculation loop for microbial spreading
Incubator



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Application

For NBB®-B:

For the production of 1 L NBB®-B mix 60 g of NBB®-P with 500 mL demineralized water and 500 mL degassed beer in a conical flask using the magnetic stirrer with the magnetic stir. Heat gently at approx. 40°C/113°F until the powder has completely dissolved. Adjust pH to 5.6-5.9 using lactic acid or NaOH. Fill the prepared NBB®-B in laboratory bottles and autoclave at 115°C/239°F for 5 min., test tubes at 115°C/239°F for 1 min. Depending on the beer, protein precipitations may occur in form of flocs or a sediment after autoclaving.

Handle the samples under sterile conditions to avoid secondary contamination.

Due to its sensitivity to high temperatures please avoid heating the NBB®-B. Use sterile test tubes with screw caps or porous stoppers (e.g. plastic-foam plugs) or sterile swing stopper sample bottles. While filling the bottles directly from the original NBB®-B flask take care to work under a microbiological workbench or close to a Bunsen burner flame to avoid a possible contamination.

For yeast analysis place 1 to 4 mL of the sample (depending on the yeast concentration) in a 50 mL swing stopper sample bottle or other suitable flask. Add 20-30 mL of NBB®-B.

Yeast samples can also be examined in a convenient way by using sterile swabs (preferably with screw caps). Place smaller yeast volumes or microbial spreading test of yeast deposits directly from several points of the yeast container (total volume ca. 0.5 mL) with a sterile swab into a swab tube. Fill the swab tube up to approx. 70% with NBB®-B and incubate (see Fig. 1). Do not seal the screw cap to firmly during incubation in order to allow CO₂ to escape from the tube. Proceed in the same way with sediments of unfiltered beer.

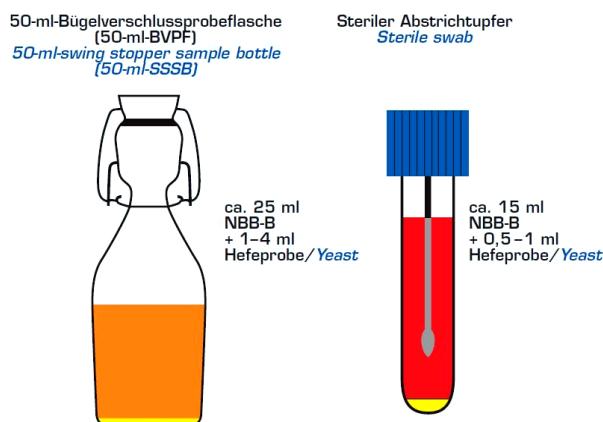


Figure 1: Preparation of samples

As an enrichment medium take a sterile swing stopper sample bottle (50 mL) or other suitable bottle and mix 2.5-10 mL NBB®-B (depending on the contamination) with the filtered beer sample to the bottle edge.



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For NBB®-A:

Dissolve 12-15 g of Agar-Agar (1.2-1.5%) in 500 mL sterile water at 95°C/203°F in an Erlenmeyer flask and subsequently add 500 mL degased beer and 60 g NBB®-P while stirring. Fill in laboratory bottles and autoclave the prepared NBB®-A at 115°C/239°F for 5-10 min.

Handle the samples under sterile conditions to avoid secondary contamination.

Liquefy the culture media in a water bath at max. 95°C/203°F. Cool it slowly for use to approx. 45°C/113°F.

For the pour plate method:

Place 3 mL of test sample in a 9 cm Petri dish and fill with warm agar to a height of 3-4 mm. Swirl the mixture gently by moving the closed Petri Dish in a rotation following a "8" -figure on the workbench.

As a Nutrient base:

Fill the nutrient media into a Petri dish to a height of 3-4 mm. Cool the dish until the NBB®-Agar solidifies and use it for:

- membrane filters by placing the filter on top of the agar
- for microbial spreading with sample swabs or inoculation loops
- for air samplers

Incubation

Incubate the test samples in NBB®-B and on NBB®-A between 26-28°C/79-82°F for 5 days. Samples not containing yeast should be incubated under anaerobic conditions.

If the samples are determined for pre-enrichment in NBB®-B for further analysis (NBB®-A or molecular biological methods such as PCR or VIT), an incubation of 2 days NBB®-B is sufficient.

Evaluation

Since the speed of the microbial growth depends on several factors (initial cell count, type, physiological condition and origin of the germs, degree of adaptation to beer), an incubation time of one day may be sufficient, in case of high contaminations, to obtain a result. Mostly several days are needed in case of trace contaminations or of very slow growing strains (e.g. *Lactobacillus lindneri*). Observe the growth-pattern during incubation time to obtain clear statements about the condition of your sample. Evaluate your samples after 5 days of incubation for a final comparability of your results.

If the contaminations are strong enough and if the organisms are typical beer spoilers, the colour of the indicator in NBB® will turn from red to yellow.

In case of trace contaminations or older, autolyzing yeast cells from the tank bottom, the indicator colour may not be evident enough to get a clear result. In this case the samples have to be examined under the microscope.



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3. Storage and Packaging Information

Packaging and Content

unit	300 g in folded box
unit size (Box)	approx. 23 cm x 11 cm x 15 cm / 9 in x 4.3 in x 5.90 in
unit gross weight (Box)	approx. 400 g/ 1 lbs.

Storage

Store at 4-8°C/40-45°F according to product specification.
Store under dry and dark conditions. Do not freeze product.

Waste Disposal

No dangerous good.
No hazardous material.
Please consider your local waste regulations.
Powder remains can be disposed of with normal laboratory waste.
Inoculated and incubated samples are to be sterilized before disposal at a temperature of 121°C/ 250°F for 20 min.

Warnings

Do not cook or freeze the culture media. Do not keep the agar liquified longer than 4 hours. Media not directly used should not be solidified again for future use. Repeated heating can restrain its functionality. The colour of the culture media is reddish and can change during storage to brownish. This is a natural phenomenon and has no impact on the functionality of the product. Always wear protective clothing when handling hot media.



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4. Related products

Sample type	Product	Format	Method	Item no.	Packaging	Incubation			Analysis
						T [°C]	t [d]	Condition	
Yeast samples Selected, harvested and brewing yeast Yeast sediments	NBB®-B	Broth in bottle	0.5-1 ml of sample + 10-20 ml of NBB®-B	2.04710.782	9 x 250 ml (glass bottle)	28°C	3-5	anaerobic	qualitative
		Broth in tubes		2.04723.646	20 x 10 ml (tube)				
Yeast-cloudy beers Green beer Unfiltrate Wheat beer	NBB®-C	Concentrated broth	95 % of sample + 5 % of NBB®-C	2.04711.782	9 x 250 ml (glass bottle)	28°C	7-14	anaerobic	qualitative
Clear beers Membrane filtered samples	NBB®-A	Agar	Filtration of 50-200 ml of sample	2.04709.782	9 x 250 ml (glass bottle)	28°C	5-7	anaerobic	quantitative
Water, rinsing water Membrane filtered samples	NBB®-A	Agar	Filtration of 50-200 ml of sample	2.04709.782	9 x 250 ml (glass bottle)	28°C	5-7	anaerobic	quantitative
Environmental air Sampling of airborne microorganism on agar plates	NBB®-A	Agar	Direct sampling	2.04709.782	9 x 250 ml (glass bottle)	28°C	5-7	anaerobic	quantitative
Surfaces in filling plants Hygiene monitoring using swabs	NBB®-B-AM	Broth	1 swab in 10 ml of NBB®-B-AM	2.04706.782	9 x 250 ml (glass bottle)	28°C	3	aerobic	qualitative
	NBB®-P	Powder	For the in-house production of NBB®-A and NBB®-B, using beer from own production.	2.04716.462	300 g (bag)				
Laboratory accessories	Smear swabs, without tube			2.04725.444	100 pc. (bag)				